References
1. Olivera-Couto A, Grana M, Harispe L, Aguilar PS (2011) The eisosome core is composed of BAR domain proteins. Mol Biol Cell 22: 2360-2372.
2. Olivera-Couto A, Aguilar PS (2012) Eisosomes and plasma membrane organization. Molecular Genetics and Genomics 287(8): 607-20.

2354-Pos Board B46

Architecture of Whole-Module and Bimodular Proteins from the 6-Deoxyerythronolide B Synthase

Andrea L. Edwards[1], Tsutomu Matsui[2], Chaitan Khosla[1].
[1]Chemistry Department, Stanford University, Stanford, CA, USA, [2]Stanford Synchrotron Radiation Lightsource, Stanford, CA, USA.

The 6-deoxyerythronolide B synthase is a prototypical assembly line polyketide synthase (PKS). Models for the quaternary structures of a full PKS module and bimodule have been derived through small-angle X-ray scattering (SAXS) analysis of a systematic set of multidomain proteins whose fragments have previously been characterized at atomic resolution. The global architectures of these proteins suggest that the ACP can interact with the KS domain through two mutually exclusive channels during polyketide chain elongation and translocation.

2355-Pos Board B47

Self-Assembley of Dehaloperoxidase-Hemoglobin Probed by Backbone Dynamics using NMR Relaxation Experiments and Molecular Dynamics Simulation

Jing Zhao[1], Mengjun Xue[2], Hanna Gracz[1], Stefan Franzen[1].
[1]North Carolina State University, Raleigh, NC, USA, [2]Technical University of Berlin, Berlin, Germany.

Dehaloperoxidase-hemoglobin (DHP) is a multi-functional protein isolated from the annelid marine worm Amphitrite ornata. It has been shown to function as an oxygen transporter, peroxidase, peroxygenase, oxidase and hydrogen sulfide oxidase with substrates that include a range of phenols and indoles. DHP primarily exists as a monomer in the solution (~90%), while is observed as a dimer in the X-ray crystal structure. The dimer-monomer equilibrium in solution has shown to be able to change the redox state of the protein which further regulates the multiple enzyme functions of DHP. The self-assembly behavior of DHP has been studied by NMR and MD simulations in terms of backbone dynamics. The R1, R2 and {1H} −15N NOE of the backbone amide N-H bonds have been measured using NMR relaxation experiments at multiple magnetic fields. The squared generalize order parameter S2 that describe the spatial restriction of the internal motions of amide N-H bond was extracted using the model-free analysis for each residue. S2 were also theoretically calculated from correlation functions based on MD simulations. The dynamic pattern of DHP monomer shows that the μs-ms slow motions experiencing by residues in the dimer interface are primarily responsible for association between each monomers. Moreover, the disulfide bond is formed between the only cysteine in DHP during the dimerization process which leads to the unique autoreduction phenomenon in DHP. While most heme proteins autooxidize, DHP will actually autoreduce if prepared in the ferric form because of its very high reduction potential (the highest known for a monomeric hemoglobin) and reduction by surface cysteines that form disulfide bonds during the dimerization process.

2356-Pos Board B48

Studying the Role of Protein Flexibility in Allosteric and Evolutionary Changes as Seen in PyrR Protein Family

Sandhya P. Tiwari[1], Tina Perica[2], Yasushi Kondo[2], Stephen McLaughlin[2], Annette Steward[3], Jane Clarke[3], Sarah A. Teichmann[4], Nathalie Reuter[1].
[1]Department of Molecular Biology, University of Bergen, Norway, Bergen, Norway, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [3]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom, [4]European Bioinformatics Institute, Hinxton, United Kingdom.

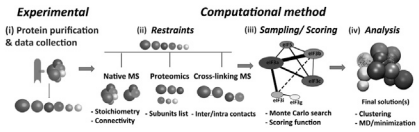
Oligomerisation is essential for the function of some proteins. The PyrR family of proteins are involved in pyrimidine operon attenuation. They are regulated by the presence of certain nucleotides such as guanosine monophosphate (GMP), which stabilises the tetrameric state. Notably, some members of this family can adopt a tetrameric oligomerisation state regardless of the presence of the GMP. Perica et al. (2012) previously found that this family, among several others, have differences in sequence outside the oligomeric interfaces and these are sufficient to explain the changes to their subunit geometry and oligomerisation states. Here, we compared the differences in structural flexibility linked to the changes in oligomeric state caused by a) specific mutations and, b) by the presence of bound GMP. We calculated the coarse-grained normal modes of dimeric units of the PyrR dimers and tetramers using an Elastic Network Model implemented in the Molecular Modelling ToolKit. We conducted several analyses including comparing the normal modes of these proteins with each other using the Bhattacharyya Coefficient similarity measure, correlations matrices, and the conformational overlap analysis, by which the contribution of these modes to the transition from one state to another can be quantified. Firstly, we found that while the dynamics were very similar between all the structures, there was a noticeable difference between the dimeric and tetrameric units. We also show that both sets of proteins transition from tetrameric to dimeric states similarly, indicating some overlap between the effects of allostery and evolution on oligomerisation in PyrR.

2357-Pos Board B49

Determining the 3D Topologies of Heteromeric Protein Assemblies by a Mass-Spectrometry Based Hybrid Approach

Argyris Politis[1,2], Carla Schmidt[1], Elina Tjioe[3], Andrej Sali[3], Carol V. Robinson[1].
[1]University of Oxford, Oxford, United Kingdom, [2]University of Ulster, Londonderry, United Kingdom, [3]University of California San Francisco, San francisco, CA, USA.

Describing, understanding, and modulating the function of the cell require elucidation of the networks and structure of its macromolecular assemblies. Here, we describe an integrative approach to determine the topologies of heteromeric assemblies using structural information derived from native mass spectrometry (MS), proteomics and chemical cross-linking MS. The method was developed and further assessed for robustness and accuracy using a benchmark of five hypothetical assemblies with simulated data and two assemblies with previously published MS data. With this benchmark in hand, we purify and characterize the yeast eukaryotic initiation factor 3 (eIF3) complex whose complete 3D topology has not been previously defined. Using a combination of MS-based data, we establish sub-stoichiometric binding of eIF5 and derive a high-likelihood structural model for the five subunit eIF3 complex. Our results further reveal two interaction modules within the eIF3 and are in accord with its role as a scaffold for other initiation factors. In conclusion, here we highlight the utility of a MS-based integrative approach for modelling complexes with unknown interactions and topology. The corresponding computational algorithm is implemented in the open source *Integrative Modeling Platform* (IMP).



2358-Pos Board B50

Native Ion Mobility-Mass Spectrometry: from Flexible Proteins to Ion Channels

Frank Sobott[1,2].
[1]Chemistry, University of Antwerp, Antwerpen, Belgium, [2]CFP-CeProMa, Center for Proteomics, Antwerpen, Belgium.

After a brief introduction into the field of native mass spectrometry and ion mobility analysis of protein complexes, we discuss how high-mass modified instrumentation can give powerful insights into the stoichiometry, subunit composition, size and shape of biomolecular particles.

We will show recent data on HFQ complexes which act as an RNA chaperone and are involved in the regulation of gene expression through facilitating post-transcriptional interactions between non-coding sRNAs and mRNA. The example of SMC proteins, which are crucial for Structural Maintenance of Chromosomes, illustrates the ability of ion mobility approaches to link information on complex assembly with the topology of the functional unit in heterogeneous systems.

Recently, we have also been studying ion channels in detergent micelles and have been able to show the gradual opening of the Mechanosensitive Channel of Large Conductance in response to the binding of a charged drug molecule inside the channel which mimics the pressure on the bulk membrane. We show that we can characterize snapshots of the gradual opening of the channel, by using collision cross sections obtained from ion mobility measurements in combination with molecular modelling.

2359-Pos Board B51

Human p52Shc Conformational Bias and Localization in c-SRC Activation

Yuko Tsutsui, Franklin A. Hays.
Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

c-SRC is a non-receptor protein tyrosine kinase that serves as an upstream regulatory molecule for a wide array of signaling cascades and proteins such as C-Jun N-terminal Kinase (JNK). Chronic JNK activation is known to alter cellular function and lead to human disease including peripheral insulin resistance in type II diabetes. Thus, modulating c-SRC activation via therapeutic intervention is a potential means for treating human disease. c-SRC is activated by saturated fatty acids and cytoplasmic regulatory proteins. One such protein, p52Shc,